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13. ABSTRACT (Maximum 200 words) Assessing ecological risk in terrestrial environments is an extraordinarily difficult, and yet to be fully-defined, task. Induced toxic effects in the ecosystem are often the result of synergistic and antagonistic interactions among a myriad of physical factors and complex mixtures of pollutants that are difficult to reproduce in the laboratory. Additionally, many pollutants are organ/system-specific in their mode of toxicity (affecting metabolism, genetic integrity, immune system function, reproduction, or some other life processes) and alterations in any one of the above physiological systems in a host organism could have important ecological consequences. Employing a single biomarker approach to risk assessment under these circumstances is largely a futile exercise. We developed an in situ multiparameter approach, incorporating a suite of acute and chronic biological indicators of exposure to lethal (population survival rates), mutagenic, immunotoxic, teratogenic, or sublethal (histopathologic, detoxication, reproductive effects) compounds, using resident small mammals to provide the robustness and sensitivity desired in an ecological risk assessment model. To characterize dose-response relationships in situ, multiparameter response profiles were quantified for cotton rats (<i>Sigmodon hispidus</i>) returned to the					
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LABORATORY. Response profiles were related to actual concentrations of contaminants in the soil (or fractions of soil) on replicated (N=12) petrochemical-contaminated and reference (N=12) locations. Sites selected for study represented a continuum (none to severe) of contaminant levels and degrees of ecotoxicity (as determined from small mammal community dynamics). We hypothesize that our mammalian multiparameter model would behave in situ in a classic dose-response fashion, mirroring the level of ecotoxicity as determined by soil analyses and ecosystem-level responses. Analysis of type and concentration of soil contaminants at each site permitted us to examine if similar response profiles can be attributed to the presence of specific contaminants that were common to all sites. Of the biomarkers employed in this study, assessing cell-mediated immunity in a lymphoproliferation assay, enumerating platelets in whole blood, assessing metabolic and phagocytic function of macrophages, and measuring myelotoxicity appeared to be the most sensitive indicators of exposure to toxicants in the soil for cotton rats, especially those from land treatment waste disposal sites. Genotoxic and pathologic indicators were not sensitive to exposure levels at these petrochemical waste sites. Tissue contaminant burdens in cotton rats were useful measures of actual metal exposure and hepatic isoenzyme activities for detoxification enzymes proved useful in assessing actual exposure to organic contaminants.

FINAL TECHNICAL REPORT

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IN SITU DOSE-RESPONSE RELATIONSHIPS FOR A MAMMALIAN MULTIPARAMETER MODEL FOR ASSESSING PETROCHEMICAL-INDUCED ECOTOXICITY

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IN SITU DOSE-RESPONSE RELATIONSHIPS FOR A MAMMALIAN MULTIPARAMETER MODEL FOR ASSESSING PETROCHEMICAL-INDUCED ECOTOXICITY

EXECUTIVE SUMMARY

Assessing ecological risk in terrestrial environments is an extraordinarily difficult, and yet to be fully-defined, task. Induced toxic effects in the ecosystem are often the result of synergistic and antagonistic interactions among a myriad of physical factors and complex mixtures of pollutants that are difficult to reproduce in the laboratory. Additionally, many pollutants are organ/system-specific in their mode of toxicity (affecting metabolism, genetic integrity, immune system function, reproduction, or some other life processes) and alterations in any one of the above physiological systems in a host organism could have important ecological consequences. Employing a single biomarker approach to risk assessment under these circumstances is largely a futile exercise. We developed an *in situ* multiparameter approach, incorporating a suite of acute and chronic biological indicators of exposure to lethal (population survival rates), mutagenic, immunotoxic, teratogenic, or sublethal (histopathologic, detoxication, reproductive effects) compounds, using resident small mammals to provide the robustness and sensitivity desired in an ecological risk assessment model. To characterize dose-response relationships *in situ*, multiparameter response profiles were quantified for cotton rats (*Sigmodon hispidus*) returned to the laboratory. Response profiles were related to actual concentrations of contaminants in the soil (or fractions of soil) on replicated ($N = 12$) petrochemical-contaminated and reference ($N = 12$) locations. Sites selected for study represented a continuum (none to severe) of contaminant levels and degrees of ecotoxicity (as determined from small mammal community dynamics). We hypothesize that our mammalian multiparameter model would behave *in situ* in a classic dose-response fashion, mirroring the level of ecotoxicity as determined by soil analyses and ecosystem-level responses. Analysis of type and concentration of soil contaminants at each site permitted us to examine if similar response profiles can be attributed to the presence of specific contaminants that were common to all sites. Of the biomarkers we employed in this study, assessing cell-mediated immunity in a lymphoproliferation assay, enumerating platelets in whole blood, assessing metabolic and phagocytic function of macrophages, and measuring myelotoxicity appeared to be the most sensitive indicators of exposure to toxicants in the soil for cotton rats, especially those from land treatment waste disposal sites. Genotoxic and pathologic indicators were not sensitive to exposure levels at these petrochemical waste sites. Tissue contaminant burdens in cotton rats were useful measures of actual metal exposure and hepatic isoenzyme activities for detoxification enzymes proved useful in assessing actual exposure to organic contaminants.

REPORT PERIOD

1 April 1995 to 31 December 1997

PRINCIPAL INVESTIGATOR

Dr. Robert L. Lochmiller

OTHER PROJECT PERSONNEL SUPPORTED

Listed below is a list of personnel that have been associated with the USAF-supported project during the life of the project; some have been supported directly while others have been associated indirectly with project as collaborators or volunteers:

Dr. Robert L. Lochmiller (PI: Professor, OSU)
 Dr. Charles W. Qualls (Co-PI: Professor, OSU)
 Dr. Karen McBee (Co-PI: Associate Professor, OSU)
 Dr. Nick Basta (Co-PI: Assistant Professor, OSU)
 Dr. Jim W. Lish (Research Assistant, 18% effort, OSU)
 Dr. William Warde (Statistical consultant, Professor, OSU)
 Soochung Kim (Ph.D. candidate: 1 April 1995 - present)
 Barbara Bowers (M.S. student: presently writing thesis)
 Dan Rafferty (M.S. candidate: 1 August 1995 - present)
 Danny Allen (Ph.D. candidate: 1 May 1996 - present)
 Jackie Schroder (M.S. candidate: soil analysis, 1 July - present)
 Lee Jones (Technician 100% effort: 1 April 1995 - present)
 Brian Faulkner (M.S. student: assisted with census, summer 1995)
 Eric Stair (M.S. student: assisted with detoxication enzyme analysis)
 Joe Roder (M.S. student: assisted with detoxication enzyme analysis)
 Mozghan Savabieasfahani (Ph.D. candidate, 1 April 1995 - present).
 Russell Pfau (Ph.D. candidate,)
 Joanna Whittier (M.S. candidate, volunteer in genetics lab)
 Nikki Manz (B.S. undergraduate assistant)
 Demetrius Mason (B.S. undergraduate assistant)
 Sundeep Chandi (recent Ph.D. graduate: manuscript preparation)
 Sabu Kuruvilla (Ph.D. candidate: assisted with necropsies and histopathology)
 Lori Gallimore, undergraduate hourly labor.
 Patrick Shinaberry, undergraduate hourly labor

RESEARCH OBJECTIVES

Our overall proposed project objective was to characterize and quantify *in situ* dose-response relationships for a multiparameter mammalian model using resident small mammals (cotton rats) inhabiting petrochemical-contaminated ecosystems. To characterize dose-response relationships *in situ*, multiparameter response profiles were statistically compared to actual concentrations of contaminants in the soil at a large

number ($N = 12$) of petrochemical-contaminated locations and reference sites ($N = 12$). We hypothesize that our mammalian multiparameter model would be sensitive in a classic dose-response fashion to the level of ecotoxicity as determined by soil analyses and ecosystem-level responses (small mammal community dynamics). Analysis of type and concentration of soil contaminants at each site would permit us to examine if similar response profiles can be attributed to the presence of specific contaminants that was common to all sites. Specifically, we performed the following tasks:

1. To select 12 distinct study sites that have a history of petrochemical contamination and possess a viable population of cotton rats. Each contaminated site was matched with an ecologically similar reference site (total of 12 reference populations) for relative comparisons of response profiles. Contaminated sites were comprised of areas with suspected low, moderate, and high levels of contamination, as judged from preliminary site remedial investigations by the EPA and Oklahoma Health Department.
2. To establish small mammal trapping grids (1 ha each) on each contaminated site and their matched reference for seasonal (winter and summer) monitoring of community structure and population demographics. Four matched areas were sampled per year over the 3-year study.
3. To conduct soil and volatile organic contaminant characterizations of each site using GC-MS analysis. Soil samples were collected to coincide with small mammal trapping sessions.
4. To quantify multiparameter response profiles (pathology, genotoxicity, immunotoxicity, metabolic toxicity) of cotton rats from petrochemical-contaminated sites ($N = 12$) compared to ecologically-matched reference sites ($N = 12$).
5. To characterize *in situ* dose-response relationships of the multiparameter model as a function of degree of toxicity using multivariate statistical analysis (multiple regression analysis and discriminant function analysis). The degree of toxicity was assessed subjectively from the nature and level of soil contaminants detected and the severity of ecosystem-level effects (community structure, population demographics) observed in the field.

RESEARCH APPROACH

Study Areas

The 12 sites of contamination selected for study consisted of disturbed terrestrial ecosystems (early seral stage plant species) that support viable populations of resident cotton rats. Each contaminated site was matched with an adjacent ecologically-similar reference site. Matched reference sites permitted us considerable experimental control over non-pollutant environmental variables (climate, nutrition, etc.), which can frequently confound interpretation of biomarker response profiles. Thus, multiparameter response profiles for cotton rats from contaminated sites were interpreted relative to their ecologically matched reference sites. The petrochemical-contaminated sites that we choose for intensive monitoring were selected from known Superfund Waste Sites and several abandoned oil refinery sites distributed throughout Oklahoma. Specific study sites were at least 1 ha in size to accommodate a sufficiently large population of cotton rats for censusing and seasonal sampling. Sites were also selected to represent varying degrees of toxicity (from low to high), based on preliminary soil and ground water contaminant analyses available from the EPA and Oklahoma Department of Health.

During year 1 of the study (summer 1995 and winter 1996) we two toxic sites located on an abandoned oil refinery in Cyril Oklahoma and consisted of a former refinery waste landtreatment (LT) facility (Cyril LT) and pond burms (PM) surrounding a former sludge pit (SP) for disposal wastes (Cyril PB SP). Two abandoned refinery sludge pits located in Cleveland and Cushing, OK referred to as Cleveland (SP) and Cushing (SP) were monitored during the same time period. All four toxic sites were matched with a reference area.

In year 2 (summer 1996 winter 1997) we evaluated two toxic sites in Oklahoma City, OK: a sludge pit on an abandoned re-refining complex formerly known as Double Eagle Oil Refinery (referred to as DBL Eagle SP); a former landfill site on Tinker Air Force Base, Oklahoma City (Tinker LF) that was scheduled for cleanup after our study, and had been used extensively in the 1950's and 1960's for the disposal of municipal waste, solvents and other aircraft maintenance waste; two contaminated sites were located 25 miles south of Tulsa OK where wastes from refineries were disposed by landtreatment (an area where only wastes had been landtreated, Tulsa SECTE LT; an area where a waste pond had been filled and capped with contaminated landtreated soil, Tulsa Cap SP/LT).

In Year 3 (summer 1997 and winter 1998) three contaminated sites were located on abandoned oil refinery, Duncan, OK and consisted of a former landtreatment facility (DuncanLF LT), an asphalt pit for acid waste sludges (DuncanTP AP), and refinery waste stream and waste sludge settling pond (DuncanPB SP). The Refinery began operation in the 1920's and shut down in the early 1980's. The fourth site was located in Ponca City, OK on an active oil refining complex where tank bottom wastes were landtreated (Conoco LT/LF).

The toxicity of each study site for quantifying dose-response relationships were described by measuring ecologically relevant endpoints and careful characterization of

the type and level of contaminants in specific fractions (supercritical fluid extracts, total, and bound) of soil samples collected from each study site and their matched reference areas. Soil samples were obtained from randomly selected locations within the boundaries of population census grids. Ecologically relevant endpoints to be measured consisted of population monitoring to quantify seasonal changes in density, survival, and recruitment, as well as, describe small mammal community structural attributes. Animals (ca. 12) from each contaminated and matched reference site were collected seasonally (summer and winter) and returned to the laboratory to fully characterize multiparameter response profiles. Response profiles were compared to the major chemical fractions in soil described above.

Data Collection

Each study population was censused and animals collected for detailed physiological assessments in both summer and winter seasons. For each seasonal assessment we collected 6 male and 6 female adult cotton rats from each population and returned them to the laboratory. Animals were processed within 48 hours of their capture from the field to minimize the chance of animals detoxifying prior to measuring selected endpoints. Multiparameter response profiles measured on each collected animal included: gross pathology, histopathology of major organs and glands, detoxification enzyme activities in liver, genotoxicity assessments, myelotoxicity assessment, and immunotoxicity assessments.

Data Analysis

Population density and survival rates were measure using program CAPTURE and Mark for cotton rats. Communities for each host population were described by measures of diversity, mean species richness, and similarity. Diversities were calculated by using the complement of Simpson's index. Comparisons of mean species richness and species diversity among treatments are in progress and are not available for the final technical report.

Differences between contaminated sites and reference sites for the suite of pathological and physiological endpoints were tested using ANOVA with season and location as main factor effects, with interaction terms. Statistical significance for all hypothesis tests was set *a priori* at $P \leq 0.05$.

RESEARCH OVERVIEW

Study Sites and Soil Contamination

Analysis of variance found metal concentrations in soil was elevated on the petrochemical sites as compared to the reference sites for several metals including: Cd ($P = 0.016$), Cr ($P = 0.003$), Cu ($P = 0.002$), Ni ($P = 0.005$), Pb ($P = 0.0002$), Sr ($P = 0.006$), Ti ($P = 0.025$), V ($P = 0.018$), and Zn ($P = 0.0001$). The mean total soil content for all the metals except Ti on the reference sites were similar to values reported for uncontaminated sites (Table 2). Duncan's multiple range test indicated that the number of sites with elevated levels varied between metals. The number of sites on which the metal level was elevated (in parenthesis) as compared to the mean of all the reference sites was Ba (3), Cd (2), Co (3), Cr (9), Cu (8), Ni (7), Pb (9), Sr (6), Ti (5), V (5), and Zn (12) (Tables 3-4). The predominant elevated metals in soils on the petrochemical sites were Cr, Cu, Ni, Pb, Sr, and Zn. Elevated levels of Cr in soil ranged from 2-fold to more than 100-fold greater than the overall mean of the reference sites. Elevated levels of Cu in soil were 2- to 85-fold greater than the overall mean of the reference sites. Elevated levels of Ni in soil were 1.5- to 3-fold greater than the overall mean of the reference sites. Elevated levels of Pb in soil were 5- to 140-fold greater than the overall mean of the reference sites. Elevated levels of Sr in soil were 2- to 20-fold greater than the overall mean of the reference sites. Elevated levels of Zn in soil were 2- to 26-fold greater than the overall mean of the reference sites. Although the sites were classified as landfarms, pond burms, and tar pits; metal contamination was randomly distributed among these three classifications.

Both the total fluoride in soil ($P = 0.001$) and HCl extractable form of fluoride ($P = 0.002$) were elevated on the petrochemical sites as compared to the reference sites. The total content of fluoride in the soil of reference sites was similar to levels from uncontaminated sites which ranges from 10 to 400 mg kg⁻¹ depending on soil texture (Table 2). Total fluoride was elevated on seven of the sites and the HCl extractable form of fluoride was elevated on nine of the petrochemical sites (Table 4). The HCl extractable form of fluoride was 4- to 25-fold greater on the elevated sites as compared to the overall mean of the reference sites. Total fluoride was 10- to 60-fold greater on the elevated sites as compared to the overall mean of the reference site. It appears that fluoride in soil is more prevalent on landfarms than on the other types of petrochemical sites.

Organic contaminants measured at petroleum contaminated sites and matched reference sites included total petroleum hydrocarbons and other semivolatiles (including PAHs). Elevated levels of organic contaminants were found above GC-MS detection limits (Table 7) at all study sites (Tables 8,9,10). Total petroleum hydrocarbon (TPH) levels were low (<1000 mg kg⁻¹) and total other semivolatile levels were low (< 500 ug kg⁻¹) at the eight sites collected in years 1995-1997 (Tables 8,9). However, soils collected from sites in the third year showed much higher levels of contamination of TPH and semivolatile organics (Table 10).

Body Tissue Contaminant Loads

Brain tissue was analyzed for organic contaminants. Only small amounts of

acenaphthene and acenaphthylene were found in brain tissue but both control and contaminated sites had similar levels of these compounds.

The overall mean content of Pb in bone was elevated ($P = 0.003$) for cotton rats collected from the petrochemical sites as compared to the reference sites. There was a significant interaction of treatment and season for Pb content ($P = 0.0175$) in cotton rat bone. Analysis using the SLICE option of the LSMEANS statement showed that that Pb levels in bone of 21.5 mg kg^{-1} were higher in cotton rats collected from the petrochemical sites in winter as compared to Pb content of 10.0 mg kg^{-1} in bone of animals collected during the summer ($P = 0.0003$). Duncan's multiple range test indicated that the number of sites with elevated levels of metal in cotton rats varied between metals. The number of sites on which the metal level was elevated (in parenthesis) as compared to the mean of all the reference sites was Ba (1), Cr (6), Pb (8), Sr (4), Ti (0), and Zn (1) (Table 5). Of the metals examined; Cr, Pb, and Sr were the most prevalent in bone tissue of cotton rats collected from the petrochemical sites. Cr content of bone were slightly elevated on some sites and were approximately 2-fold greater than the overall mean of bone Cr in cotton rats collected from the reference sites. The elevated concentrations of Pb in bone were approximately 2- to 42-fold greater than the overall mean of cotton rats collected from the reference sites. The elevated concentrations of Sr in bone were only slightly elevated and were approximately 1.5-fold greater than the overall mean of cotton rats collected from the reference sites. The overall mean content of fluoride in bone was elevated ($P = 0.004$) for cotton rats collected from the petrochemical sites as compared to the reference sites. There was a significant interaction of treatment and season for fluoride content ($P = 0.0377$) in cotton rat bone. Analysis using the SLICE option of the LSMEANS statement showed that that fluoride levels of 1926 mg kg^{-1} in bone were higher in cotton rats collected from the petrochemical sites in winter as compared to fluoride content of 788 mg kg^{-1} in bone of animals collected during the summer ($P = 0.0001$). Fluoride concentrations in bone of cotton rats collected from the reference sites were similar to levels reported in other small mammal studies on uncontaminated sites. Fluoride content of bone was also elevated on seven of the petrochemical sites as compared to the overall mean of the reference sites (Table 5). Elevated fluoride concentrations in bone were approximately 5- to 23-fold greater than the overall mean of cotton rats collected from the reference sites.

Although elevated levels of metal were found in both soils and cotton rats from the petrochemical sites, there was not a strong relationship between metal content of bone and soil metal concentrations. (Table 6). However, there was a strong relationship between bone fluoride and HCl extractable fluoride and total forms of fluoride in soil. The soil Pb in our study covered a small range. Perhaps relationships between soil concentrations of Pb and bone Pb may be difficult to determine when relatively small ranges of soil contamination are examined.

Pathology

Gross examinations have proven extremely useful for determination of pathologic, toxicologic, preneoplastic and carcinogenic alterations in cotton rats. Necropsies included visual evaluation of the entire carcass, including teeth, and

weights of liver, kidney, adrenal, and gonads; and liver volume. Special attention was given teeth; upper and lower incisors were scored for color and enamel integrity. Liver, kidney, adrenal, pancreas, representative intestinal areas, heart, lung, and brain were selectively removed and placed in neutral buffered formalin and processed for histological examination; blood was collected for hematological analysis and serum chemistries.

We observed significant ($P < 0.05$) differences in relative mass of the liver on contaminated landfarm sites compared to their matched reference sites. Increases in relative liver mass have been reported in laboratory mice exposed to petrochemical-contaminated soil (Silkworth et al. 1984). Rattner et al. (1993) observed elevated relative liver mass in cotton rats from the MOTCO Inc. waste site in Texas, which was consistent with exposure to petroleum hydrocarbons. However, at an arsenic-contaminated site, Rattner et al. (1993) observed reductions in the relative mass of the liver in cotton rats. We observed that relative live mass was elevated on two landfarm sites yet reduced on another, suggesting that different contaminants were responsible for these disparate results.

Kidney mass was lower in cotton rats from contaminated landfarm sites in summer, whereas relative kidney mass was both reduced (two sites) and increased (one site). This inconsistency is also suggestive of differing forms of contamination and toxicity across land treatment units. The trends in relative liver and kidney masses were similar in our study, suggesting that contaminants in the soil effected these two organ systems in a similar fashion.

The prevalence of dental fluorosis in this study was somewhat less in that approximately 50% of the cotton rats captured on the seven petrochemical sites with elevated levels of soil and bone fluoride displayed dental lesions (severity score ≥ 3). The majority ($> 99\%$) of the cotton rats collected from the reference sites in this study did not have dental lesions. Severity of dental lesions varied from site to site and ranged from a score of one (slight striation in lower incisor) to a score of five (white chalky lower and upper incisors). Overall approximately 80% of the cotton rats collected from the seven petrochemical sites with elevated levels of soil and bone fluoride had some form of dental lesions (severity score of 1 to 5). The prevalence of dental fluorosis was approximately 50% higher in winter than in summer animals. Dental lesions were more prevalent on sites A, C, D, and L than on the other sites. However, more than 50% of the cotton rats collected from sites B, E, and H had lesions. Regression analysis revealed a strong relationship ($P = 0.0001$) between incisor score and fluoride content in bone of cotton rats. However, a more detailed analysis using Fisher's exact test indicated that the severity of dental fluorosis could not always be accurately predicted by the concentration of fluoride in bone. By classifying total content of fluoride in bone as low ($< 1000 \text{ mg kg}^{-1}$), medium (≥ 1000 but $< 3000 \text{ mg kg}^{-1}$), or high ($\geq 3000 \text{ mg kg}^{-1}$) and ranking dental lesions in cotton rats as low (< 3) or high (≥ 3), it was possible to determine whether fluoride content in bone could predict the severity of dental fluorosis in cotton rats. The analysis revealed that only 5% of the cotton rats had a high severity score when bone fluoride concentrations are less than 1000 mg kg^{-1} . Thus, low levels of bone fluoride can accurately predict the severity of dental fluorosis. Approximately 52% of the animals collected had a high

severity score when bone fluoride ranged from 1000 to 3000 mg kg⁻¹. Medium levels of fluoride in bone could not be used to predict the severity of dental fluorosis. At bone fluoride levels greater than 3000 mg kg⁻¹, approximately 78% of the rats had a high severity score. Therefore, high levels of bone fluoride can accurately predict the severity of dental fluorosis.

Myelotoxicity

The hematopoietic system is uniquely sensitive to a wide variety of toxic agents and environmental pollutants. Evidence has accumulated that exposure to certain environmental chemicals can produce myelotoxicity in laboratory animals at low dose levels where other manifestations of toxicity are not observed in the parenchymal organs. Evidence suggests that suppression of granulocyte-macrophage progenitors is demonstrated at lower-level exposures to contaminants. Bone marrow, with rapidly renewing cell population, is one of the most sensitive endpoints for detecting health effects of environmental contaminants because alterations in bone marrow progenitors occur at exposure levels where only minimal or no parenchymal organ toxicity is seen. Examination of colony formation of the hematopoietic cells following exposure to chemicals has proven to be a very sensitive indicator of myelotoxicity, often being suppressed prior to detecting hematological changes, as well as a means for mechanistic study of the toxicity of various drugs. In order to form large colonies of differentiating macrophages and/or granulocytes, colony stimulating factor (CSF) is necessary for the *in vitro* proliferation of bone marrow progenitor cells of macrophages and/or granulocytes. It has been reported that the injection of mice with the bacterial lipopolysaccharide (LPS) elicits acute rises in serum CSF levels.

Since we did not observe any parenchymal organ toxicity from the histopathology examinations of cotton rats, we examined altered patterns of progenitor cell proliferation and differentiation in bone marrow hematopoiesis by *in vitro* colony growth assays. We verified a significant decrease in CFU-GM in cotton rats exposed to cyclophosphamide under controlled laboratory conditions before using the technique to assess animals from the field populations. CFU-GM colony formation was suppressed in rats from petrochemical waste sites (overall means ranged from 61.14% to 74.94%) compared to cotton rats from reference sites (100%), and the inhibition of colony formation was statistically significant from reference values during all collections except one winter. Whether the observed changes were sufficient in magnitude to affect disease resistance following exposure to the toxic insults was not determined.

Monoxygenase Activity

Liver samples were processed within 2 min. of sacrifice for use in detoxication enzyme assays. Evaluation of hepatic cytochrome P-450 induction in wild hispid cotton rats has been suggested as a useful endpoint for biological monitoring of various environmental contaminants (Elangbam et al. 1989). Hepatic microsomes contain multiple cytochrome P-450 isoenzymes that possess broad substrate selectivity. The different isoenzymes function in the activation and detoxification of various xenobiotics. O-dealkylation of resorufin ethers (induction of the CYP1A subfamily [classic inducers are 3-methylcholanthrene, β -naphthoflavone] has been shown to be particularly useful

in assessing xenobiotic exposure in wild rodents (Lubet et al. 1985). We examined the response and sensitivity of hepatic cytochrome P-450 isoenzyme activities of the cotton rat inhabiting petrochemical-contaminated environments containing complex mixtures of organic hydrocarbons and heavy metals. We hypothesized that feral cotton rats would be sensitive to contaminant exposure as reflected by elevated hepatic microsomal cytochrome P-450 activity and associated O-dealkylation activities for ethoxyresorufin and methoxyresorufin.

Analyses of these data from cotton rats inhabiting three reference sites and three contaminated sites at an abandoned oil refinery Superfund waste site in Oklahoma revealed several important differences. O-dealkylation of ethoxyresorufin and methoxyresorufin was significantly greater (170 – 180%) in cotton rats from contaminated sites compared to those from reference sites in summer, but not winter. These results indicate that the cotton rat may be a sensitive model species for biomonitoring petrochemical-contaminated ecosystems and demonstrate the importance of multi-season sampling in biomonitoring studies.

Immunotoxicity

The immune system was assessed completely for any evidence of immunotoxicity by assessing macrophage function, immune organ development and cellularity, serum antibody levels, innate immunity, in vivo cell-mediated immunity, lymphoproliferative responsiveness of cultured lymphocytes, and natural killer cell function (Tables 11ab, 12ab, 13ab, and 14ab).

Cotton rats collected from contaminated landfarm sites generally showed an enhanced lymphoproliferative response following stimulation with the plant-lectin Con-A. This assay is useful for assessing the ability of mature and immature T-cells to undergo blastogenesis following antigenic stimulation. Benzo (a) pyrene at low concentrations (10^{-5}M – 10^{-8}M) is capable of enhancing the proliferative response of mouse splenocytes following in vitro stimulation with Con-A and PHA (Tomar 1991). Constan et al. (1995) noted a significant increase in hepatocyte proliferation in vivo for F344 rats following long-term exposures to low levels of a complex petrochemical mixture containing arsenic, benzene, chloroform, chromium, lead, phenol, and trichloroethylene.

The macrophage arm of the nonspecific immune system has been consistently shown to be responsive to many forms of immunotoxicants under laboratory exposure conditions (Descotes 1988). We observed both quantitative and qualitative differences in indices of nonspecific immunity in the cotton rat. Total cell yields from the peritoneal cavity, including numbers of recovered macrophages, was frequently elevated in animals from contaminated landfarm sites. Measurements of integrity of the respiratory burst via mitochondrial reduction of NBT showed a trend comparable to that for total cell yield in cotton rats, suggesting exposure caused some up-regulation of macrophage activity. Exposure to metals such as chromium, copper, and manganese can be associated with similar numerical responses in macrophages of laboratory rodent models. Wojdani and Alfred (1984) observed that several PAHs were capable of inducing substantial elevations in macrophage yields in a dose-dependent fashion. Elevated phagocytic activity and H_2O_2 production by mouse macrophages have been

observed following exposures to low concentrations of lead and cadmium (Cd, 3.0 mg kg⁻¹ food; Pb 1.5 mg kg⁻¹ food; Baykov et al. 1996).

Cotton rats collected from contaminated landfarm sites experienced a marginal depression in their hypersensitivity responsiveness to an intradermal challenge of PHA, suggesting that some functional suppression of cell-mediated immunity may have resulted from exposure to the complex mixtures of contaminants on these sites. McMurry (1993) and Propst et al. (1995) showed a similar depression for *in vivo* response to antigenic challenge with PHA for cotton rats collected from petrochemical-contaminated sites. This type of hypersensitivity reaction is mediated by macrophages and involves T-cells that produce lymphokines in response to the PHA. Laboratory studies have documented dysregulation of skin immune function through loss of Langerhan cells when mice were exposed to 7,12 dimethylbenz[a]anthracene (Halliday 1988). However, the elevated yields and metabolic activity of macrophages that we observed in cotton rats from contaminated sites would seem to suggest that the reduced response to PHA challenge may be more T-cell dependent.

The results of this study indicate that the petrochemical wastes that were applied to soils have no uniform immunomodulatory effect on cotton; immune alterations were sometimes indicative of enhancement while on other sites these same assays were indicative of suppression of the immune response. These observations are not unexpected given the considerable diversity of contaminants present in the soils of the five different land treatment facilities we investigated. Many contaminants such as metals are well known for their differing abilities to either enhance or suppress immune responses. Waste products disposed of through land application technologies such as these vary from one industrial site to another. For example, land treatment unit 3 was used almost exclusively for the disposal of tank-bottom wastes, while land treatment 1 was used for the disposal of waste sludges from sedimentation ponds as well as tank-bottom wastes. An additional factor contributing to the observed differences in response variables is the length of time wastes were actually applied to the soils. Most of these sites lacked historical records on what was applied and how long the landfarms were in operation. Of the assays we employed in this study, assessing cell-mediated immunity in a lymphoproliferation assay, enumerating platelets, and assessing macrophage function appeared to be the most sensitive indicators of exposure for cotton rats from land treatment sites.

Genotoxicity Assessments:

Bone marrow metaphase chromosomal spreads were prepared and scored for the presence of six classes of chromosomal damage. For the Fall 1995 trapping period, mean number of lesions per cell ranged from 0.03 (Cleveland Refinery Toxic Site, and Reference Site 1) to 0.16 (Cyril Refinery Toxic Site). Chromatid breaks were the most frequently observed class of aberration and ranged from a mean of 1.27 (Cyril Reference Site 1) to 3.17 (Cyril Refinery Toxic site 2). During the Spring 1996 trapping period, mean number of lesions per cell were consistently much lower at all sites and ranged from 0.004 at Cleveland Refinery Toxic Site to 0.023 at Cyril Refinery Toxic Site 2. Levels of damage observed in all classes of aberrations were also consistently lower during the Spring 1996 trapping period with chromatid breaks again being the

most frequently observed class of damage followed by acentric fragments. The two highest values for mean number of chromatid breaks were from the two Cyril Refinery Toxic Sites. Although only preliminary, these data suggest that the Superfund Site at Cyril, OK, which was initially considered to be the most heavily contaminated of our sites, consistently showed the most severe response at the chromosomal level.

Spleen tissue was also analyzed using Flow Cytometry for non-cell lethal genetic lesions, which can be transmitted to daughter cells gradually leading to increased dispersion of nuclear DNA content among progeny cells. Statistical analyses for the Fall 1995 collecting period indicated that the highest CVs occurred at Cyril Refinery Toxic Site 1, and the lowest CV occurred at Cyril Reference Site 2, again suggesting that animals from the Cyril Superfund site are suffering the most serious genetic impact as measured by flow cytometry. A proliferation index (PI) was calculated for each animal from toxic sites by adding the percent cells in S and G2/M stages of the cell cycle then dividing this number by the same value calculated for all matched reference site animals (values close to 1 indicates a normal proliferating cell population). For the Fall 1995 trapping period, animals from Cyril Refinery Toxic Sites 1 and 2 had PIs of 1.54 and 0.76, suggesting deviation from normal DNA synthesis rates. Values for the Spring 1996 trapping period showed a similar trend, again suggesting that the Cyril Superfund Site was the most severely impacted site based on genetic endpoints.

Overview of Findings

Soils of petrochemical sites were contaminated with Cd, Cr, Cu, Ni, Pb, Sr, Ti, V, and Zn. Metal contamination was randomly distributed among landfarms, pond burms, and tar pits. Fluoride in soil was elevated (10- to 60-fold greater) on the petrochemical sites as compared to the reference sites and was more prevalent on landfarms. Fluoride and Pb were also elevated in bone tissue of cotton rats collected from the petrochemical sites as compared to the reference site. Lead levels in bone of 21.5 mg kg^{-1} were higher in cotton rats collected from the petrochemical sites in winter as compared to Pb of 10.0 mg kg^{-1} in bone of animals collected during the summer. Most cotton rats (80%) collected from seven petrochemical sites with elevated levels of soil and bone fluoride had dental fluorosis. The prevalence of dental fluorosis was 50% higher in winter than in summer animals. There was a strong relationship ($r = 0.85$) between bone fluoride and total content of fluoride in soil.

The results of this study indicate that the petrochemical wastes that were applied to soils have no uniform effect on small mammals across all study areas. For example, immunomodulatory effects on cotton rats were sometimes indicative of enhancement while on other sites these same assays were indicative of suppression of the immune response. These observations are not unexpected given the considerable diversity of contaminants present in the soils of the five different land treatment facilities we investigated. Many contaminants such as metals are well known for their differing abilities to either enhance or suppress immune responses. Waste products disposed of through land application or other disposal technologies vary from one industrial site to another. For example, land treatment unit 3 was used almost exclusively for the disposal of tank-bottom wastes, while land treatment 1 was used for the disposal of

waste sludges from sedimentation ponds as well as tank-bottom wastes. An additional factor contributing to the observed differences in response variables is the length of time wastes were actually applied to the soils. Most of these sites lacked historical records on what was applied and how long. Of the assays we employed in this study, assessing cell-mediated immunity in a lymphoproliferation assay, enumerating platelets, assessing macrophage function, and myelotoxicity appeared to be the most sensitive indicators of exposure to immunotoxicants for cotton rats from land treatment sites. Genotoxic and pathologic indicators were not sensitive to exposure levels at these petrochemical waste sites. Tissue contaminant burdens in cotton rats were useful measures of actual metal exposure and hepatic isoenzyme activities for detoxification enzymes proved useful assessing actual exposure to organic contaminants.

To prevent accumulation of contaminants in cotton rats, land application of petrochemical wastes should be based on inorganic contaminants. Wastes that contain excessive levels of inorganic contaminants may not be suitable for land application.

RESULTING CREATIVE ACCOMPLISHMENTS ON THE PROJECT

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Table 1. Description of petrochemical contaminated soils.

Site	Type	Soil pH	Soil OC ^a	Soil Texture	Soil EC ^b
A	landfarm	7.5	3.2	loam	0.24
B	landfarm	6.6	4.7	loam	0.21
C	landfarm	6.5	4.7	loam	0.19
D	landfarm	7.0	6.5	sandy loam	0.27
E	landfarm	6.9	14.5	sandy loam	0.32
F	pond burm	7.1	7.9	loam	0.20
G	pond burm	6.8	3.9	loam	0.16
H	pond burm	5.1	33.8	loamy sand	0.18
I	tar pit	6.0	3.3	silt loam	0.13
J	tar pit	7.0	3.4	loam	0.23
K	tar pit	6.6	3.4	clay loam	0.21
L	tar pit	6.5	30.4	sandy loam	0.18

^aorganic carbon content in %

^belectrical conductivity (dS m⁻¹)

Table 2. Comparison of range and mean metal content of study site with baseline soils.

Metal	Petroleum sites ^a	Reference sites	Baseline soils
Ba	83-312 (211)	16.0-883 (196)	100-3000 ^b (580)
Cd	0.10-5.12 (0.96)	0.00-0.60 (0.25)	0.00-0.61 ^c (0.22)
Co	3.78-12.30 (8.82)	3.6-17.5 (7.94)	6.3-30.3 ^c (14.0)
Cr	7.70-1863 (267)	3.9-52.6 (18.3)	5.0-1500 ^b (54.0)
Cu	16.8-1210 (152)	5.3-74.0 (14.2)	2.7-23.9 ^c (10.5)
Ni	12.4-50.6 (29.2)	5.8-28.6 (15.5)	6.1-41.7 ^c (21.0)
Pb	20.9-1679 (410)	4.1-29.8 (12.0)	5.1-27.2 ^c (16.5)
Sr	16.7-390 (86.3)	9.2-47.6 (18.2)	10.0-500 ^b (67.0)
Ti	9.23-223 (73.0)	5.4-228 (51.3)	684-4081 ^c (2765)
V	11.8-95.7 (42.8)	4.9-50.7 (21.2)	3.8-81.0 ^c (31.7)
Zn	58.3-894 (208)	12.9-51.6 (34.9)	22.3-127.3 ^c (31.7)
Hcl F	2.0-1026 (247)	0.6-26.5 (4.03)	-----
Fusion F	60.2-5257 (1748)	10.9-217 (89.7)	10.0-400 ^d (360)

^a Range and mean (in parenthesis) metal content of soils

^b Adriano 1986

^c Basta et al. 1998

^d Kabata-Pendias and Pendias 1984

Table 3. Total mean concentrations of metals and fluoride in soils from petrochemical sites. All values are in mg kg⁻¹ on a soil basis. Bolded values are greater (p< 0.05) than the mean of all reference sites. Values with the same letter are not significantly different.

Site	Ba	Cd	Co	Cr	Cu	Ni	Pb
a	206 bcd	0.48 bcd	6.82 de	233 b	36.5 cde	50.6 ab	61.1 ef
b	193 bcd	0.32 bcd	17.8 a	52.8 de	18.5 fg	31.1 abcd	20.9 h
c	160 bcdef	0.38 bcd	11.2 bc	105 c	24.8 defg	19.6 bcdef	29.1 fgh
d	273 abc	0.33 bcd	9.80 bcd	292 b	102 b	27.7 abcd	1240 a
e	312 ab	2.38 a	7.30 de	1863 a	1210 a	38.7 a	1679 a
f	169 cdef	0.48 bcd	3.78 g	423 b	195 b	12.4 f	769 b
g	191 bcde	0.73 b	9.78 bcd	7.7 i	16.8 fg	14.9 def	343 bc
h	161 cdef	0.23 cd	7.43 de	95.9 cd	54.4 cd	35.8 ab	243 bcd
I	212 bcdef	0.32 bcd	4.68 fg	13.1 hi	51.0 cd	19.8 cdef	24.2 gh
j	82.9 f	0.70 bc	8.49 cde	26.3 fg	68.9 bc	26.3 abcde	170 de
k	483 a	5.12 a	12.3 b	37.5 ef	18.1 efg	32.0 abc	147 efg
l	87.4 ef	0.10 d	6.45 ef	54.0 de	30.3 cdef	42.0 ab	198 cde
Reference	196 def	0.25 bcd	7.94 de	18.3 gh	14.2 g	15.5 ef	12.0 h

Table 4. Total mean concentrations of metals and fluoride in soils from petrochemical sites. All values are in mg kg⁻¹ on a soil basis. Bolded values are greater ($p < 0.05$) than the mean of all reference sites. Values with the same letter are not significantly different.

Site	Sr	Ti	V	Zn	HCl F	Fusion F
A	192 b	164 ab	92.4 a	173 bc	732 a	2672 bc
B	19.2 f	19.2 f	23.0 c	90.9 de	338 b	878 d
C	23.5 def	23.5 ef	23.1 c	259 b	1026 a	4316 ab
D	74.8 c	124 abc	70.2 ab	215 b	344 b	5257 a
E	390 a	223 a	40.8 b	894 a	22.2 d	2082 bc
F	158 c	31.0 ef	14.0 de	83.8 de	20.5 de	64.7 g
G	25.1 def	25.1 ef	8.1 e	249 b	6.23 fg	103 efg
H	50.3 d	104 bc	93.9 a	96.3 de	124 c	3213 bc
I	37.6 de	50.8 de	11.8 de	87.8 de	2.04h	60.2 fg
J	16.7 f	9.23 g	17.1 cd	140 cd	16.1 ef	169 e
K	26.8 def	26.8 ef	22.9 c	153 de	4.37 gh	150 ef
L	21.3 ef	74.9 cd	95.7 a	58.3 e	332 b	2016 cd
Reference	18.2 f	51.3 ef	21.2 cd	34.9 f	4.03 gh	89.7 efg

Table 5. Mean concentration of bone in cotton rats collected from petrochemical sites.
 All values are in mg kg⁻¹ of bone. Bolded values are greater (p<0.05) than the control.
 Values with the same letter are not significantly different

Site	Ba	Cr	Pb	Sr	Ti	Zn	F
A	29.5 ef	2.9 b	4.6 c	239 ab	0.5 a	179 b	1515 bc
B	45.6 cd	1.4 cd	1.4 def	134 e	0.3 ab	184 ab	1610 bc
C	40.2 de	0.5 d	0.7 f	133 e	0.2 b	177 b	2964 a
D	65.5 bc	2.9 ab	63.4 a	145 de	0.3 ab	185 ab	830d
E	61.9 bc	3.2 a	12.8 b	174 cd	0.3 ab	167 bc	1733 c
F	31.1 ef	0.4 d	12.4 b	212 bc	0.5 ab	170 bc	89.5 f
G	47.2 cd	0.8 cd	60.7 a	132 e	0.4 ab	180 b	171 e
H	79.4 b	2.7 ab	2.2 def	134 e	0.3 ab	172 bc	2671 b
I	81.5 b	0.7 d	3.5 cd	257 a	0.5 ab	150 c	137 e
J	21.3 f	3.7 ab	3.8 c	83.5 f	0.4 ab	163 bc	172.6 e
K	126 a	1.3 cd	3.0 cde	163 cde	0.2 b	211 a	137.5 e
L	78.4 b	2.9 ab	20.1 b	134 e	0.3 ab	197 b	3683a
Reference	105 b	1.6c	1.5 ef	148 e	0.4ab	173 bc	159 e

Table 6. Simple correlation between bone and soil contents.

	Ba	Cr	Pb	Sr	Ti	Zn	HCl F	Total F
r	-0.00	0.30	0.36	0.40	0.05	-0.07	0.70	0.85
p-value	1.00	0.34	0.25	0.21	0.89	0.84	0.02	0.00

Table 7. Detection limits for organic contaminants by GC-MS. Results are expressed as ug kg⁻¹ soil.

Organic Contaminant	Detection Limit	Organic Contaminant	Detection Limit
Acenaphthene	10	2,4-Dinitrotoluene	100
Acenaphthylene	10	2,6-Dinitrotoluene	100
Acetophenone	100	Diphenylamine	100
4-Aminobiphenyl	100	Diphenylhydrazine	100
Aniline	100	Di-n-octyl phthalate	100
Anthracene	10	Fluoranthene	10
Benzidine	100	Fluorene	10
Benzoic Acid	100	Hexachlorobenzene	100
Benzo (a) anthracene	10	Hexachlorobutadiene	100
Benzo (b and k) fluoranthene	10	Hexachlorocyclopentadiene	100
Benzo (g,h,i,) perylene	10	Hexachloroethane	100
Benzo (a) pyrene	10	Indeno (1,2,3) pyrene	10
Benzyl alcohol	100	Isophorone	100
bis (2-Chloro ethoxy) methane	100	3-methylcholanthrene	100
bis (2-Chloroethyl) ether	100	2-Methylnaphthylene	100
bis (2-Chloroisopropyl) ether	100	2-Methylphenol	100
bis (2-ethylhexyl) phthalate	100	3 or 4-Methylphenol	100
4-Bromophenyl-phenylether	100	Napthalene	10
Butylbenzylphthalate	100	1-Napthylamine	100
4-Chloroaniline	100	2-Napthylamine	100
4-Chloro-3-methylphenol	100	2-Nitroaniline	100
1-Chloronapthalene	100	3-Nitroaniline	100
2-Chloronapthalene	100	4-Nitroaniline	100
2-Chlorophenol	100	Nitrobenzene	100
4-Chlorophenyl-phenylether	100	2-Nitrophenol	100
Chrysene	10	4-Nitrophenol	100
Dibenzo (a,j) acridine	100	N-Nitrosodi-n-butylamine	100
Dibenz (a,h) anthracene	10	N-Nitrosodimethylamine	100
Dibenzofuran	100	N-Nitroso-di-n-propylamine	100
Di-n-butyl phthalate	100	N-Nitrosodiphenylamine	100
1,2-Dichlorobenzene	100	N-Nitrosopiperidine	100
1,3-Dichlorobenzene	100	Pentachlorobenzene	100
1,4-Dichlorobenzene	100	Pentachloronitrobenzene	100
3,3 Dichlorobenzidine	100	Pentachlorophenol	100
2,4-Dichlorophenol	100	Phenacetin	100
2,6-Dichlorophenol	100	Phenanthrene	10
Diethyl phthalate	100	Phenol	100
p-Dimethylaminoazobenzene	100	2-Picoline	100
7,12-Dimethylbenz(a)anthracene	100	Pronamide	100
a,a-Dimethylphenethylamine	100	Pyrene	10
2,4-Dimethylphenol	100	1,2,4,5-Tetrachlorobenzene	100
Dimethylphthalate	100	2,3,4,6-Tetrachlorophenol	100
4,6-Dinitro-2-methylphenol	100	1,2,4-Trichlorobenzene	100
2,4-Dinitrophenol	100	2,4,5-Trichlorophenol	100
		2,4,6-Trichlorophenol	100

Table 8. Total petroleum hydrocarbon, semivolatile priority pollutants including polyaromatic hydrocarbons in soils from petrochemical contaminated sites. Values are means of six samples, values in parentheses are means from reference sites. Study sites for 1995-6.

Organic Chemical Contaminant	Cyril Landfarm Site A	Cyril Pondberms Site F	Cushing Site J	Cleveland Site I
Total Petroleum Hydrocarbons	275 (30.3)	645 (71.7)	mg kg⁻¹ 295 (27.0)	65.9 (31.3)
Napthalene	0 (0)	19.1 (0)	ug kg⁻¹ 9.0 (0)	0 (0)
Acenaphthylene	0 (0)	0 (0)	0 (0)	0 (0)
Acenaphthene	0 (0)	0 (0)	12.3 (0)	0 (0)
Fluorene	0 (0)	0 (0)	13.0 (0)	0 (0)
Phenanthrene	12.8 (0)	135 (8.8)	140 (0)	149 (3.6)
Anthracene	9.9 (0)	333 (0)	38.2 (0)	24.8 (0)
Fluoranthene	0 (9.7)	25.3 (0)	123 (0)	63.5 (0)
Pyrene	7.5 (0)	171 (0)	133 (0)	128 (18.8)
Benzo (a) anthracene	4.5 (0)	68.5 (0)	111 (0)	133 (15.8)
Chrysene	11.3 (0)	132 (9.8)	441 (0)	209 (19.1)
Benzo (b and k) fluoranthene	0 (7.1)	63.8 (10.8)	188 (5.5)	202 (34.9)
Benzo (a) pyrene	8.3 (0)	57.9 (0)	114 (6.8)	106 (37.8)
Indeno (1,2,3-cd) pyrene	0 (0)	17.4 (0)	45.8 (0)	24.2 (0)
Dibenz (a,h) anthracene	15.0 (0)	54.2 (0)	13.6 (0)	0 (0)
Benzo (g,h,i) perylene	46.8 (0)	163 (0)	108 (0)	55.6 (0)
Bis (2-ethylhexyl) phthalate	41.6 (0)	65.7 (82.0)	0 (0)	0 (0)
Di-n-butylphthalate	89.5 (0)	130 (116)	56.7 (0)	38.4 (0)
Diethylphthalate	85.7 (0)	52.3 (0)	24.2 (0)	0 (0)
2,6-dinitrotoluene	0 (0)	170 (0)	0 (0)	0 (0)
Butylbenzylphthalate	42.5 (0)	17.3 (0)	0.0 (0)	0 (0)
2-methylnaphthylene	0 (0)	41.7 (0)	0 (0)	0 (0)

Table 9. Total petroleum hydrocarbon, semivolatile priority pollutants including polyaromomatic hydrocarbons in soils from petrochemical contaminated sites. Values are means of six samples, values in parentheses are means from reference sites. Study sites for 1996-7.

Contaminant	Double Eagle Site G	Tinker Site K	Mounds Site C	Tulsa Cap Site B
Total Petroleum Hydrocarbons	89.3 (2.0)	17.9 (2.0)	mg kg⁻¹ 609.9 (6.1)	769.7 (0)
Napthalene	0.0 (0)	17.9 (0)	ug kg⁻¹ 290 (0)	92.0 (0)
Acenaphthylene	2.6 (0)	17.9 (0)	54.0 (0)	0.0 (0)
Acenaphthene	0.0 (0)	18.5 (0)	19.9 (0)	0.0 (0)
Fluorene	0.0 (0)	19.1 (0)	28.3 (0)	3.5 (0)
Phenanthrene	22.0 (0)	19.8 (0)	217 (0)	131 (0)
Anthracene	2.5 (0)	20.5 (0)	79.1 (0)	25.2 (0)
Fluoranthene	67.1 (9.7)	21.2 (0)	8.3 (0)	4.4 (0)
Pyrene	34.4 (0)	22.0 (0)	35.4 (0)	41.6 (0)
Benzo (a) anthracene	15.3 (0)	21.8 (0)	19.3 (0)	31.6 (0)
Chrysene	29.5 (0)	21.8 (0)	44.2 (0)	60.8 (0)
Benzo (b and k) fluoranthene	38.4 (7.3)	22.2 (0)	32.4 (0)	48.3 (0)
Benzo (a) pyrene	4.9 (0)	22.3 (0)	39.0 (0)	47.8 (0)
Indeno (1,2,3-cd) pyrene	19.9 (0)	21.3 (0)	63.2 (0)	29.7 (0)
Dibenz (a,h) anthracene	0.0 (0)	21.5 (0)	0.0 (0)	51.9 (26.7)
Benzo (g,h,i) perylene	28.7 (0)	21.8 (0)	419 (0)	281 (0)
Bis (2-ethylhexyl) phthalate	0 (0)	23.0 (0)	90.0 (0)	105 (0)
Di-n-butylphthalate	345 (127)	23.3 (0)	568 (276)	871 (35.3)
Diethylphthalate	0 (0)	9.1 (0)	125.3 (0)	0.0 (0)
Butylbenzylphthalate	0 (0)	0.3 (0)	0.0 (0)	0.0 (0)
2-methylnaphthylene	0 (0)	0.3 (0)	1119 (0)	504 (0)

Table 10. Total petroleum hydrocarbon, semivolatile priority pollutants including polyaromomatic hydrocarbons in soils from petrochemical contaminated sites. Values are means of six samples. Values in parentheses are means from reference sites. Study sites for 1996-7.

Contaminant	Duncan Landfarm Site D	Conoco Site E	Duncan Pond Burm, Site H	Duncan Tar Pit, Site L
Total Petroleum Hydrocarbons	3240 (38.4)	5670 (9.2)	mg kg⁻¹ 2530 (28.0)	774 (0)
Napthalene	758	755	ug kg⁻¹ 1150	1017
Acenaphthylene	0 (0)	0 (0)	0 (0)	0 (0)
Acenaphthene	0 (0)	0 (0)	0 (0)	0 (0)
Fluorene	0 (0)	37 (0)	0 (0)	0 (0)
Phenanthrene	0 (0)	2320 (0)	2063 (0)	2113 (0)
Anthracene	0 (0)	0 (0)	713 (0)	833 (0)
Fluoranthene	0 (0)	432 (0)	1525 (0)	500 (0)
Pyrene	0 (0)	1550 (0)	1833 (0)	2100 (0)
Benzo (a) anthracene	750	1920 (0)	1820 (0)	3388 (0)
Chrysene	0 (0)	2127 (0)	3330 (0)	3988 (0)
Benzo (b and k) fluoranthene	0 (0)	1380 (0)	2300 (0)	2638 (0)
Benzo (a) pyrene	0 (0)	0 (0)	3163 (0)	5175 (0)
Indeno (1,2,3-cd) pyrene	0 (0)	0 (0)	0 (0)	0 (0)
Dibenz (a,h) anthracene	0 (0)	0 (0)	0 (0)	0 (0)
Benzo (g,h,i) perylene	0 (0)	0 (0)	0 (0)	0 (0)
Bis (2-ethylhexyl) phthalate	0 (0)	0 (0)	0 (0)	0 (0)
Di-n-butylphthalate	0 (0)	0 (0)	0 (0)	0 (0)
Diethylphthalate	0 (0)	0 (0)	0 (0)	0 (0)
Butylbenzylphthalate	0 (0)	0 (0)	0 (0)	0 (0)
2-methylnapthylene	0 (0)	0 (0)	0 (0)	0 (0)

Table 11a. Hematological parameters of adult cotton rats collected from reference and toxic sites in Oklahoma, USA during September 1995 through March 1998.

LOCATION	TYPE	DATE	SEASON	Site	WBC			RBC			HGB			HCT			MCV			MCH			MCHC			PLT		
					n	X	stderr	n	X	stderr	n	X	stderr	n	X	stderr	n	X	stderr	n	X	stderr	n	X	stderr	n	X	stderr
CYRIL	LT	10/3/95	SUMMER	R	10	22.7	3.0	11	6.4	0.2	11	14.0	0.3	11	41.6	0.8	11	65.5	0.8	11	22.0	0.3	11	33.5	0.2	11	610	55
CYRIL	LT	10/3/95	SUMMER	T	11	19.6	2.0	12	6.3	0.1	12	14.3	0.4	12	42.7	1.3	12	67.4	0.9	12	22.6	0.3	12	33.6	0.2	12	652	66
CYRIL	LT	2/21/96	WINTER	R	11	13.5	1.8	12	5.7	0.3	12	12.2	0.5	12	36.2	1.5	12	64.3	0.8	12	21.7	0.4	12	33.8	0.4	12	386	39
CYRIL	LT	2/21/96	WINTER	T	11	15.3	1.4	12	6.0	0.1	12	13.1	0.2	12	38.7	0.8	12	64.6	0.5	12	21.9	0.2	12	33.8	0.1	12	466	30
CYRIL PB	SP	9/29/95	SUMMER	R	10	14.7	1.1	12	6.5	0.2	12	13.7	0.3	12	41.2	1.0	12	63.9	0.7	12	21.3	0.4	12	33.3	0.3	12	523	49
CYRIL PB	SP	9/29/95	SUMMER	T	11	14.2	1.1				12	14.3	0.3	11	42.6	1.1	11	67.5	0.6	11	22.6	0.3	11	33.5	0.2	11	585	49
CYRIL PB	SP	3/8/96	WINTER	R	11	7.5	0.9	12	5.8	0.2	12	12.6	0.3	12	37.1	1.0	12	63.7	0.8	12	21.6	0.4	12	33.9	0.2	12	317	23
CYRIL PB	SP	3/8/96	WINTER	T	10	10.4	3.0	12	5.4	0.2	12	11.8	0.3	12	35.1	1.1	12	65.6	1.3	12	22.2	0.5	12	33.8	0.3	11	371	20
CLEVELAND	SP	9/21/95	SUMMER	R	11	16.7	1.9	12	53.6	48.0	12	10.9	0.4	12	36.2	1.3	12	65.0	0.8	12	19.6	0.3	12	30.2	0.3	12	615	56
CLEVELAND	SP	9/21/95	SUMMER	T	10	16.7	1.6	12	6.0	0.1	12	11.8	0.3	12	38.6	0.8	12	64.5	1.0	12	19.7	0.4	12	30.5	0.3	12	536	28
CLEVELAND	SP	3/2/96	WINTER	R	10	8.7	0.9	12	6.3	0.2	12	12.7	0.2	12	38.4	0.8	12	61.0	0.9	12	20.2	0.3	12	33.2	0.2	12	346	20
CLEVELAND	SP	3/2/96	WINTER	T	10	11.1	1.0	12	6.0	0.1	12	12.4	0.1	12	37.0	0.4	12	61.4	0.7	12	20.5	0.3	12	33.4	0.2	12	400	22
CUSHING	SP	9/25/95	SUMMER	R	10	17.2	1.4	12	6.1	0.2	12	13.3	0.4	12	39.5	1.3	12	64.7	1.0	12	21.7	0.4	12	33.6	0.3	12	560	37
CUSHING	SP	9/25/95	SUMMER	T	12	18.3	2.4	12	5.7	0.1	12	12.3	0.3	12	36.6	0.9	12	64.6	0.6	12	21.7	0.2	12	33.6	0.2	12	552	35
CUSHING	SP	2/26/96	WINTER	R	10	13.7	2.2	12	6.0	0.3	9	12.9	1.1	12	38.6	1.9	12	64.0	0.7	9	21.0	1.1	9	32.8	1.5	12	388	40
CUSHING	SP	2/26/96	WINTER	T	11	13.7	1.3	12	6.0	0.4	12	12.5	0.5	12	37.4	1.8	12	63.1	1.6	12	21.3	0.8	12	33.7	0.4	12	452	29
TINKER	LF	9/17/96	SUMMER	R	9	15.0	1.5	12	6.0	0.3	11	13.0	0.8	12	39.3	2.2	12	65.5	1.0	11	21.4	0.4	11	32.5	0.3	12	481	39
TINKER	LF	9/17/96	SUMMER	T	8	16.5	1.9	12	5.9	0.3	10	13.2	0.7	12	39.1	1.8	12	66.6	1.0	10	22.2	0.5	10	33.2	0.3	12	567	49
TINKER	LF	2/16/97	WINTER	R	9	12.8	1.7	12	6.8	0.1				12	42.0	0.8	12	62.1	0.6							12	537	31
TINKER	LF	2/16/97	WINTER	T	9	15.4	1.5	11	6.7	0.2				11	40.0	0.6	11	59.6	0.8	1	19.6		1	32.3		11	477	25
DBL EAGLE	SP	9/21/96	SUMMER	R	9	13.6	1.6	12	6.3	0.2	12	13.7	0.3	12	41.7	0.9	12	66.0	0.8	12	21.7	0.3	12	32.9	0.2	12	595	61
DBL EAGLE	SP	9/21/96	SUMMER	T	11	18.2	3.0	12	6.3	0.2	12	13.6	0.3	12	41.3	1.1	12	65.3	0.7	12	21.6	0.3	12	33.0	0.2	11	661	46
DBL EAGLE	SP	2/22/97	WINTER	R	10	17.5	1.9	12	6.2	0.1	12	13.2	0.2	12	38.8	0.7	12	62.8	0.8	12	21.3	0.4	12	34.0	0.2	12	444	35
DBL EAGLE	SP	2/22/97	WINTER	T	10	20.5	1.6	12	6.2	0.1	12	13.4	0.2	12	39.6	0.5	12	63.7	0.5	12	21.5	0.2	12	33.8	0.2	12	390	22

LT= Landtreatment
 SP=Sludge Pit
 LF= Landfill
 AP=Asphalt Pit
 R=Reference Site
 T =Toxic Site
 WBC= White Blood Cells
 RBC= Red Blood Cells
 HGB= Hemoglobin
 HCT= Hematocrit
 MCV= Mean Corpuscular volume
 MCH= Mean Corpuscular Hemoglobin
 MCHC= Mean Corpuscular Hemoglobin Concentration
 PLT= Platelets

Table 11b. Hematological parameters of adult cotton rats collected from reference and toxic sites in Oklahoma, USA during September 1995 through March 1998.

LOCATION	TYPE	DATE	SEASON	Site	WBC			RBC			HGB			HCT			MCV			MCH			MCHC			PLT										
					thsn/	cu mm	n	stdev	n	cu mm	n	stdev	n	grams/	dl	n	stdev	n	%	n	stdev	n	pico	grams	n	stdev	n	%	thsn/	cu mm	n	stdev	n	cu mm	n	stdev
TULSA SECT E	LT	9/29/96	SUMMER	R	9	23.4	5.1	12	6.2	0.1	11	13.2	0.2	12	40.1	0.7	12	65.2	0.8	11	21.5	0.3	11	32.9	0.2	0										
TULSA SECT E	LT	9/29/96	SUMMER	T	9	20.2	2.5	12	6.7	0.1	12	13.1	0.3	12	39.7	0.9	12	59.6	0.6	12	19.6	0.2	12	33.0	0.2	0										
TULSA SECT E	LT	2/26/97	WINTER	R	5	62.6	9.1	12	6.2	0.1	5	12.9	0.3	12	38.3	0.8	12	61.9	0.7	7	24.1	2.0	5	34.6	0.2	12	550	34								
TULSA SECT E	LT	2/26/97	WINTER	T	8	54.5	8.1	12	6.4	0.1	9	13.0	0.3	12	38.9	0.6	12	61.2	0.7	9	20.6	0.3	9	33.6	0.2	12	681	23								
TULSA CAP	SP/LT	9/25/96	SUMMER	R	10	23.5	1.5	12	6.7	0.2	12	13.8	0.4	12	41.7	1.2	12	62.3	0.7	12	20.6	0.4	12	33.1	0.3	0										
TULSA CAP	SP/LT	9/25/96	SUMMER	T	10	25.5	3.8	12	6.5	0.2	12	13.3	0.3	12	39.9	0.8	12	61.1	0.8	12	20.4	0.3	12	33.3	0.3	0										
TULSA CAP	SP/LT	3/2/97	WINTER	R	10	19.3	1.9	12	6.0	0.1	12	12.4	0.2	12	37.4	0.4	12	62.3	0.4	12	20.7	0.2	12	33.3	0.1	12	403	29								
TULSA CAP	SP/LT	3/2/97	WINTER	T	8	26.5	4.7	12	6.4	0.2	12	13.0	0.3	12	39.0	1.0	12	61.1	0.7	12	20.4	0.3	12	33.4	0.2	12	507	22								
DUNCAN LF	LT	9/20/97	SUMMER	R	9	22.1	2.8	12	5.8	0.2	12	12.9	0.3	12	38.4	1.1	12	66.4	0.6	12	22.3	0.2	12	33.7	0.2	12	610	40								
DUNCAN LF	LT	9/20/97	SUMMER	T	11	23.1	2.7	12	6.3	0.2	12	13.6	0.3	12	40.5	0.9	12	64.5	0.6	12	21.7	0.2	12	33.7	0.3	12	462	32								
DUNCAN LF	LT	2/17/98	WINTER	R	11	16.3	1.6	12	6.3	0.2	12	13.3	0.2	12	70.4	30.3	12	63.8	0.7	12	21.4	0.3	12	33.4	0.1	12	361	31								
DUNCAN LF	LT	2/17/98	WINTER	T	9	19.1	4.7	12	6.1	0.2	12	12.6	0.2	12	69.2	31.0	12	62.9	0.5	12	20.7	0.3	12	32.9	0.2	12	414	34								
DUNCAN TP	AP	9/16/97	SUMMER	R	10	23.8	3.6	12	6.7	0.1	12	14.6	0.2	11	44.3	0.9	10	66.3	0.8	10	22.0	0.2	10	33.2	0.3	10	514	55								
DUNCAN TP	AP	9/16/97	SUMMER	T	8	23.9	5.3	11	6.5	0.2	11	14.4	0.5	11	43.4	1.5	11	66.8	1.0	10	22.2	0.5	10	33.3	0.4	11	564	29								
DUNCAN TP	AP	2/25/98	WINTER	R	11	21.3	3.9	12	6.3	0.1	12	13.3	0.2	12	39.8	0.6	12	63.3	0.9	12	21.2	0.3	12	33.4	0.2	12	364	30								
DUNCAN TP	AP	2/25/98	WINTER	T	6	18.5	4.1	10	6.4	0.2	10	13.3	0.3	10	39.8	1.0	10	62.1	0.7	10	20.8	0.3	10	33.4	0.2	10	517	31								
DUNCAN PB	SP	9/24/97	SUMMER	R	11	19.0	2.8	12	6.1	0.2	12	13.2	0.2	12	39.8	0.7	12	65.5	1.2	12	21.7	0.5	12	33.1	0.3	12	483	35								
DUNCAN PB	SP	9/24/97	SUMMER	T	12	16.7	2.1	12	6.5	0.1	12	14.4	0.3	12	43.2	1.1	12	66.9	0.6	12	22.3	0.2	12	33.3	0.2	12	547	52								
DUNCAN PB	SP	2/21/98	WINTER	R	9	22.0	3.8	12	5.7	0.1	12	12.1	0.2	12	36.5	0.5	12	63.9	1.0	12	21.2	0.4	12	33.2	0.2	12	350	23								
DUNCAN PB	SP	2/21/98	WINTER	T	11	18.7	2.3	12	6.1	0.1	12	13.5	0.2	12	40.0	0.6	12	65.3	0.5	12	22.0	0.2	12	33.7	0.1	12	483	38								
CONOCO	LT/LF	9/28/97	SUMMER	R	11	18.6	2.8	11	6.7	0.1	11	14.5	0.2	10	43.1	0.4	10	64.6	0.9	11	21.7	0.3	11	33.6	0.1	11	406	21								
CONOCO	LT/LF	9/28/97	SUMMER	T	10	20.1	2.2	11	6.6	0.1	11	14.4	0.2	11	43.2	0.7	10	65.3	0.9	10	21.7	0.4	11	33.2	0.2	11	502	47								
CONOCO	LT/LF	3/1/98	WINTER	R	11	20.1	3.0	12	5.7	0.2	12	12.7	0.4	12	38.6	1.3	12	67.5	0.8	12	22.2	0.4	12	32.9	0.3	12	388	36								
CONOCO	LT/LF	3/1/98	WINTER	T	9	29.6	3.0	12	57.4	51.6	12	12.9	0.3	12	39.0	0.9	12	66.9	0.8	12	22.2	0.3	12	33.2	0.2	12	474	34								

LT= Landfill treatment
 SP= Sludge Pit
 LF= Landfill
 AP= Asphalt Pit
 R= Reference Site
 T= Toxic Site
 WBC= White Blood Cells
 RBC= Red Blood Cells
 HGB= Hemoglobin
 HCT= Hematocrit
 MCV= Mean Corpuscular volume
 MCH= Mean Corpuscular Hemoglobin
 MCHC= Mean Corpuscular Hemoglobin Concentration
 PLT= Platelets

Table 12a. Body weight and selected immune organ and cellularity parameters of adult cotton rats collected from reference and toxic sites in Oklahoma, USA during September 1995 through March 1998.

LOCATION	TYPE	DATE	SEASON	SITE	Body Weight			Popliteal Lymph Node			Popliteal Lymph Node Count			Adrenal Glands (Paired)			Spleen			Splenocytes		
					n	x	stdev	n	x	stdev	n	x	stdev	n	x	stdev	n	x	stdev	n	wt (g)	n
CYRIL	LT	10/9/95	SUMMER	R	11	126.2	6.6	11	0.0187	0.0037	11	4.0	0.6	11	0.0540	0.0043	11	0.3329	0.0463	11	0.1060	0.0190
CYRIL	LT	10/9/95	SUMMER	T	12	146.1	10.5	12	0.0219	0.0036	12	6.7	1.4	12	0.0613	0.0070	12	0.4125	0.0451	12	0.1377	0.0180
CYRIL	LT	2/21/96	WINTER	R	12	92.7	4.8	12	0.0081	0.0015	12	2.9	0.5	12	0.0387	0.0029	12	0.1677	0.0133	12	0.3286	0.0230
CYRIL	LT	2/21/96	WINTER	T	12	122.7	7.2	12	0.0115	0.0021	12	4.5	1.0	12	0.1416	0.0022	12	0.1987	0.0185	12	0.3229	0.0380
CYRIL PB	SP	9/29/95	SUMMER	R	12	131.7	9.9	12	0.0177	0.0037	12	4.7	1.2	12	0.0634	0.0070	12	0.2665	0.0283	12	0.2788	0.0152
CYRIL PB	SP	9/29/95	SUMMER	T	12	133.2	7.0	12	0.0240	0.0073	12	8.2	2.9	12	0.0526	0.0043	12	0.3361	0.0290	12	0.1827	0.0376
CYRIL PB	SP	3/8/96	WINTER	R	12	89.2	5.2	12	0.0080	0.0020	12	2.9	0.4	12	0.0378	0.0028	12	0.1585	0.0211	12	0.3543	0.0299
CYRIL PB	SP	3/8/96	WINTER	T	12	109.9	6.2	12	0.0075	0.0005	12	2.7	0.2	12	0.0433	0.0052	12	0.2216	0.0288	12	0.3477	0.0274
CLEVELAND	SP	9/21/95	SUMMER	R	12	135.9	10.2	12	0.0174	0.0019	12	4.0	0.7	12	0.0633	0.0056	12	0.2371	0.0214	12	0.2406	0.0315
CLEVELAND	SP	9/21/95	SUMMER	T	12	144.1	9.4	12	0.0248	0.0047	12	5.9	1.2	12	0.0600	0.0058	12	0.3052	0.0486	12	0.1804	0.0330
CLEVELAND	SP	3/2/96	WINTER	R	12	92.2	4.4	11	0.0107	0.0021	11	6.1	1.4	12	0.0403	0.0030	12	0.1177	0.0157	12	0.3690	0.0272
CLEVELAND	SP	3/2/96	WINTER	T	12	81.3	5.2	11	0.0094	0.0031	11	4.0	0.9	12	0.0331	0.0022	12	0.1281	0.0133	12	0.3601	0.0388
CUSHING	SP	9/25/95	SUMMER	R	12	126.1	8.5	12	0.0140	0.0023	12	4.7	0.8	12	0.0628	0.0087	12	0.1876	0.0275	12	0.2055	0.0232
CUSHING	SP	9/25/95	SUMMER	T	12	111.3	7.7	12	0.0228	0.0063	12	7.2	2.1	12	0.0533	0.0049	12	0.1992	0.0235	12	0.2178	0.0462
CUSHING	SP	2/26/96	WINTER	R	12	72.9	5.7	12	0.0140	0.0061	12	3.6	1.3	12	0.0766	0.0465	12	0.1050	0.0215	12	0.3492	0.0476
CUSHING	SP	2/26/96	WINTER	T	12	84.1	8.1	12	0.0061	0.0013	12	1.8	0.4	12	0.0379	0.0039	12	0.1829	0.0477	12	0.3582	0.0414
TINKER	LF	9/17/96	SUMMER	R	12	141.5	9.9	12	0.0149	0.0034	12	5.3	1.8	12	0.0504	0.0054	12	0.2544	0.0243	12	0.2414	0.0264
TINKER	LF	9/17/96	SUMMER	T	12	148.6	11.8	12	0.0154	0.0025	12	4.5	0.8	12	0.0520	0.0049	12	0.3212	0.0262	12	0.2323	0.0281
TINKER	LF	2/18/97	WINTER	R	12	76.3	8.2	12	0.0037	0.0004	12	1.5	0.2	12	0.0275	0.0032	12	0.1072	0.0140	12	0.2518	0.0207
TINKER	LF	2/18/97	WINTER	T	12	97.3	7.4	12	0.0056	0.0011	12	2.5	0.5	12	0.0362	0.0037	12	0.1386	0.0170	11	0.2894	0.0306
DBL EAGLE	SP	9/21/96	SUMMER	R	12	138.9	8.6	12	10.0111	9.9989	12	4.4	0.8	12	0.0546	0.0060	12	0.2561	0.0205	11	0.3150	0.0290
DBL EAGLE	SP	9/21/96	SUMMER	T	12	152.4	11.4	12	0.0143	0.0038	12	5.5	1.5	12	0.0625	0.0082	12	0.3258	0.0482	12	0.2467	0.0364
DBL EAGLE	SP	2/22/97	WINTER	R	12	80.7	6.7	12	0.0051	0.0008	12	2.6	0.4	12	0.0308	0.0026	12	0.0931	0.0177	11	0.2505	0.0322
DBL EAGLE	SP	2/22/97	WINTER	T	12	82.9	3.8	12	0.0033	0.0004	12	1.4	0.2	12	0.0287	0.0013	12	0.1000	0.0110	11	0.2623	0.0224

LT= Landtreatment
SP=Sledge Pit
LF= Landfill
AP=Asphalt Pit
R=reference site
T = toxic site

Table 12b. Body weight and selected immune organ and cellularity parameters of adult cotton rats collected from reference and toxic sites in Oklahoma, USA during September 1995 through March 1998.

LOCATION	TYPE	DATE	SEASON	SITE	Body Weight			Popliteal Lymph Node (Paired)			Popliteal Lymph Node Count			Adrenal Glands (Paired)			Spleen wt			Splenocytes $\times 10^6$ /mg spleen		
					n	X	stderr	n	X	stderr	n	X	stderr	n	X	stderr	n	X	stderr	n	X	stderr
TULSA SECT E	LT	9/29/96	SUMMER	R	12	122.8	7.6	11	0.0171	0.0022	12	5.9	1.1	12	0.0474	0.0039	12	0.2223	0.0223	12	0.2242	0.0259
TULSA SECT E	LT	9/29/96	SUMMER	T	12	102.1	6.0	12	0.0145	0.0015	12	5.3	0.8	12	0.0476	0.0047	12	0.1664	0.0178	12	0.3144	0.0271
TULSA SECT E	LT	2/28/97	WINTER	R	12	70.3	3.3	12	0.0094	0.0020	12	51.2	14.4	12	0.0283	0.0021	12	0.0888	0.0120	12	0.3204	0.0283
TULSA SECT E	LT	2/28/97	WINTER	T	12	75.7	3.7	12	0.0085	0.0026	12	52.8	18.6	12	0.0255	0.0014	12	0.1080	0.0067	12	0.3698	0.0470
TULSA CAP	SP/LT	9/25/96	SUMMER	R	12	124.5	7.0	12	0.0168	0.0052	12	7.1	2.2	12	0.0570	0.0058	12	0.2306	0.0237	12	0.3067	0.0154
TULSA CAP	SP/LT	9/25/96	SUMMER	T	12	120.7	7.8	12	0.0156	0.0020	12	7.2	1.4	12	0.0529	0.0056	12	0.2268	0.0104	12	0.3848	0.0471
TULSA CAP	SP/LT	3/2/97	WINTER	R	12	82.5	5.1	12	0.0062	0.0007	12	2.9	0.3	12	0.0290	0.0021	12	0.1361	0.0187	12	0.3074	0.0331
TULSA CAP	SP/LT	3/2/97	WINTER	T	12	81.9	5.0	12	0.0081	0.0023	12	3.8	0.9	12	0.0278	0.0022	12	0.1117	0.0139	12	0.3714	0.0243
DUNCAN LF	LT	9/20/97	SUMMER	R	12	151.2	8.8	12	0.0273	0.0065	12	11.0	3.1	12	0.0590	0.0038	12	0.3290	0.0269	12	0.2125	0.0258
DUNCAN LF	LT	9/20/97	SUMMER	T	12	136.7	6.9	12	0.0138	0.0015	12	5.2	0.8	12	0.0478	0.0051	12	0.2675	0.0293	11	0.2144	0.0285
DUNCAN LF	LT	2/17/98	WINTER	R	12	80.7	4.9	12	0.0094	0.0018	12	3.9	0.7	12	0.0210	0.0020	12	0.0981	0.0167	12	0.2455	0.1211
DUNCAN LF	LT	2/17/98	WINTER	T	12	83.1	5.9	12	0.0107	0.0018	12	4.6	0.9	12	0.0254	0.0026	11	0.1100	0.0121	12	0.1424	0.0128
DUNCAN TP	AP	9/16/97	SUMMER	R	12	131.1	7.0	12	0.0231	0.0036	12	6.4	1.4	12	0.0445	0.0046	12	0.2817	0.0264	12	0.3064	0.0299
DUNCAN TP	AP	9/16/97	SUMMER	T	12	129.9	8.9	12	0.0139	0.0026	11	4.4	1.2	11	0.0499	0.0059	11	0.2468	0.0246	11	0.3615	0.0365
DUNCAN TP	AP	2/25/98	WINTER	R	12	81.2	4.2	12	0.0128	0.0034	12	4.7	1.5	12	0.0318	0.0015	12	0.1199	0.0157	12	0.2883	0.0293
DUNCAN TP	AP	2/25/98	WINTER	T	12	83.2	4.4	12	0.0121	0.0026	12	4.0	0.6	12	0.0305	0.0020	12	0.1093	0.0106	12	0.2888	0.0366
DUNCAN PB	SP	9/24/97	SUMMER	R	12	109.7	5.2	12	0.0165	0.0020	12	5.2	0.8	12	0.0452	0.0038	12	0.2512	0.0290	12	0.3955	0.0512
DUNCAN PB	SP	9/24/97	SUMMER	T	12	159.2	12.5	12	0.0155	0.0026	12	5.7	1.3	12	0.0219	0.1156	12	0.3103	0.0254	12	0.3000	0.0305
DUNCAN PB	SP	2/21/98	WINTER	R	12	83.4	2.8	12	0.0117	0.0038	12	3.6	1.0	12	0.0246	0.0015	12	0.1162	0.0116	12	0.3790	0.0290
DUNCAN PB	SP	2/21/98	WINTER	T	12	92.2	5.1	12	0.0083	0.0013	12	3.6	0.7	12	0.0357	0.0027	12	0.1404	0.0190	12	0.4043	0.0480
CONOCO	LT/LF	9/28/97	SUMMER	R	12	127.9	9.3	12	0.0127	0.0018	12	3.6	0.6	12	0.0516	0.0066	12	0.2022	0.0206	12	0.3007	0.0462
CONOCO	LT/LF	9/28/97	SUMMER	T	12	145.0	12.5	12	0.0225	0.0051	12	5.7	2.1	12	0.0626	0.0083	12	0.3647	0.0578	12	0.3069	0.0194
CONOCO	LT/LF	3/1/98	WINTER	R	12	89.3	5.5	12	0.0080	0.0013	12	3.2	0.6	12	0.0330	0.0025	11	0.1236	0.0227	12	0.2065	0.0367
CONOCO	LT/LF	3/1/98	WINTER	T	12	92.0	5.4	12	0.0097	0.0029	12	4.0	1.5	12	0.0295	0.0024	12	0.1432	0.0149	12	0.2987	0.0341

LT= Landtreatment
SP=Sludge Pit
LF= Landfill
AP=Asphalt Pit
R=reference site
T = toxic site

Table 13a. Total peritoneal macrophages, macrophage differential, and macrophage metabolic activity of adult cotton rats collected from reference and toxic sites in Oklahoma, USA during September 1995 through March 1998.

LOCATION	TYPE	DATE	SEASON	SITE	Total Macrophages						Differential Peritoneal Macrophage Count												Macrophage Metabolic activity (nm)					
					x10 ⁶						% Macrophages				% Mast Cells				% Neutrophils				% Lymphocytes				activity (nm)	
					n	X	stdev	n	X	stdev	n	X	stdev	n	X	stdev	n	X	stdev	n	X	stdev	n	X	stdev	n	X	stdev
CYRIL	LT	10/3/95	SUMMER	R	11	32.6	8.3	11	81	3.4	11	0.0	0.0	11	0.4	0.2	11	19	4.1	10	0.035	0.011						
CYRIL	LT	10/3/95	SUMMER	T	12	37.6	9.0	12	80	3.5	12	0.0	0.0	12	0.1	0.1	12	20	3.5	12	0.061	0.018						
CYRIL	LT	2/21/96	WINTER	R	12	6.9	0.8	12	79	3.3	12	0.2	0.1	12	0.0	0.0	12	21	3.4	12	0.075	0.026						
CYRIL	LT	2/21/96	WINTER	T	12	12.4	2.1	12	75	2.0	12	0.4	0.2	12	0.0	0.0	12	23	2.1	12	0.091	0.042						
CYRIL PB	SP	9/29/95	SUMMER	R	12	5.8	0.8	12	59	4.3	12	0.4	0.2	12	0.0	0.0	12	41	4.2	12	0.053	0.007						
CYRIL PB	SP	9/29/95	SUMMER	T	12	4.3	0.6	12	62	2.1	12	0.5	0.5	12	0.0	0.0	12	38	2.1	12	0.054	0.009						
CYRIL PB	SP	3/8/96	WINTER	R	12	13.5	2.9	12	79	3.1	12	0.2	0.1	12	0.5	0.2	12	20	3.1	12	0.031	0.009						
CYRIL PB	SP	3/8/96	WINTER	T	12	17.2	3.4	12	79	3.1	12	0.7	0.3	12	0.5	0.2	12	19	3.3	11	0.031	0.007						
CLEVELAND	SP	9/21/95	SUMMER	R	12	4.2	0.6	12	64	4.2	12	0.6	0.3	12	0.0	0.0	12	35	4.2	12	0.092	0.036						
CLEVELAND	SP	9/21/95	SUMMER	T	12	3.5	0.6	12	61	4.1	12	0.5	0.2	12	0.0	0.0	12	38	4.2	12	0.066	0.015						
CLEVELAND	SP	3/2/96	WINTER	R	12	7.7	0.7	12	82	1.8	12	0.5	0.3	12	0.2	0.1	12	18	1.7	12	0.105	0.007						
CLEVELAND	SP	3/2/96	WINTER	T	12	5.9	0.9	12	80	2.6	12	1.3	0.3	12	0.3	0.1	12	18	2.6	12	0.114	0.021						
CUSHING	SP	9/25/95	SUMMER	R	12	4.2	1.0	12	62	4.2	12	0.4	0.2	12	0.0	0.0	12	37	4.3	12	0.013	0.003						
CUSHING	SP	9/25/95	SUMMER	T	12	3.0	0.5	12	74	2.4	12	0.6	0.2	12	0.0	0.0	12	25	2.4	11	0.015	0.004						
CUSHING	SP	2/26/96	WINTER	R	12	6.8	1.2	12	82	3.3	12	0.8	0.3	12	0.0	0.0	12	17	3.5	12	0.044	0.016						
CUSHING	SP	2/26/96	WINTER	T	12	8.9	1.4	12	87	2.0	12	0.8	0.5	12	0.0	0.0	12	12	2.0	12	0.030	0.007						
TINKER	LF	9/17/96	SUMMER	R	12	21.3	2.9	12	74	3.2	12	0.0	0.0	12	0.0	0.0	12	26	3.2	12	0.030	0.005						
TINKER	LF	9/17/96	SUMMER	T	12	13.0	1.6	12	68	2.4	12	0.2	0.1	12	0.0	0.0	12	32	2.3	12	0.063	0.017						
TINKER	LF	2/18/97	WINTER	R	12	6.3	1.0	12	91	3.9	12	0.5	0.3	12	0.0	0.0	12	9	4.2	12	0.275	0.022						
TINKER	LF	2/18/97	WINTER	T	12	5.3	0.6	12	94	0.9	12	0.9	0.3	12	0.0	0.0	12	5	0.8	11	0.243	0.017						
DBL EAGLE	SP	9/21/96	SUMMER	R	12	16.5	3.4	12	75	7.0	11	0.0	0.0	11	0.0	0.0	12	18	2.6	12	0.044	0.008						
DBL EAGLE	SP	9/21/96	SUMMER	T	11	22.2	3.0	11	85	2.6	11	0.0	0.0	11	0.0	0.0	11	15	2.6	11	0.049	0.007						
DBL EAGLE	SP	2/22/97	WINTER	R	12	7.5	1.0	12	94	0.7	12	0.8	0.2	11	0.2	0.2	12	5	0.8	12	0.216	0.014						
DBL EAGLE	SP	2/22/97	WINTER	T	12	8.8	1.6	12	95	0.8	12	0.6	0.2	10	0.2	0.2	12	3	0.4	12	0.223	0.015						

LT= Landtreatment
SP=Sludge Pit
LF= Landfill
AP=Asphalt Pit
R=reference site
T = toxic site

Table 13b. Total peritoneal macrophages, macrophage differential, and macrophage metabolic activity of adult cotton rats collected from reference and toxic sites in Oklahoma, USA during September 1995 through March 1998.

LOCATION	TYPE	DATE	SEASON	SITE	Total Macrophages										Differential Peritoneal Macrophage Count										Macrophage Metabolic activity (nm)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
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 R=reference site
 T = toxic site

Table 14a. Delayed type hypersensitivity, splenic natural killer cell activity (apoptosis, % specific lysis), and splenocyte proliferation following stimulation with Con-A, PWM, and IL-2 of adult cotton rats collected from reference and toxic sites in Oklahoma, USA during September 1995 through March 1998.

LOCATION	TYPE	DATE	SEASON	SITE	DTH		NKcell		NKcell		Slim Ind.		Splenocyte Proliferation		Slim Ind.		Slim Ind.					
					n	X	Absolute Difference	NKcell Apoptosis Avg DPM	NKcell Apoptosis % specific lysis	Con-A		PWM		IL-2								
										n	stderr	n	stderr	n	stderr	n	stderr	n	stderr			
CYRIL	LT	10/3/95	SUMMER	R	10	0.087	0.008	11	55817	6970	11	51.1	6.1	10	1.9	0.5	10	2.0	0.6	10	2.0	0.5
CYRIL	LT	10/3/95	SUMMER	T	12	0.085	0.009	12	58933	5585	12	48.4	4.9	12	3.0	1.2	12	2.2	0.8	12	4.4	1.6
CYRIL	LT	2/21/96	WINTER	R	12	0.102	0.008	12	7181	760	12	61.2	4.1	12	7.0	0.9	12	19.2	5.0	12	21.6	4.2
CYRIL	LT	2/21/96	WINTER	T	12	0.118	0.008	12	5822	726	12	68.6	3.9	12	8.3	1.3	12	14.7	3.8	12	20.0	2.3
CYRIL PB	SP	9/29/95	SUMMER	R	12	0.095	0.017	12	20515	1448	12	49.1	3.6	12	8.4	1.6	12	6.5	2.4	12	6.9	0.8
CYRIL PB	SP	9/29/95	SUMMER	T	12	0.087	0.012	12	18634	1751	12	53.8	4.3	12	8.9	2.2	12	6.5	1.4	12	6.7	1.0
CYRIL PB	SP	3/8/96	WINTER	R	11	0.090	0.007	12	7238	959	12	66.3	4.5	12	51.3	4.3	12	26.0	6.0	12	41.0	4.3
CYRIL PB	SP	3/8/96	WINTER	T	11	0.095	0.006	12	8169	1368	12	62.0	6.4	12	48.6	7.6	12	40.5	10.3	12	32.2	5.0
CLEVELAND	SP	9/21/95	SUMMER	R	12	0.084	0.012	12	82815	6714	12	43.0	4.6	12	10.1	2.0	12	13.2	2.9	12	11.4	3.5
CLEVELAND	SP	9/21/95	SUMMER	T	12	0.090	0.009	12	84940	6352	12	41.8	4.3	12	7.0	1.1	12	15.5	2.8	12	17.4	3.4
CLEVELAND	SP	3/2/96	WINTER	R	12	0.078	0.006	12	10428	1123	12	52.6	5.1	12	57.6	10.8	12	117.2	15.3	12	58.9	6.6
CLEVELAND	SP	3/2/96	WINTER	T	12	0.098	0.006	12	9769	1078	12	55.6	4.9	12	45.0	9.8	12	73.2	18.0	12	38.3	5.2
CUSHING	SP	9/25/95	SUMMER	R	12	0.122	0.011	12	53116	3196	12	46.2	3.2	12	12.0	1.9	12	22.4	4.3			
CUSHING	SP	9/25/95	SUMMER	T	12	0.126	0.014	11	48401	5376	11	50.9	5.4	12	9.2	1.9	12	12.1	3.6			
CUSHING	SP	2/26/96	WINTER	R	12	0.104	0.004	12	13678	1540	12	54.7	5.1	12	10.5	2.4	12	37.1	14.5	12	20.6	5.2
CUSHING	SP	2/26/96	WINTER	T	12	0.088	0.007	11	11994	2106	11	60.3	7.0	12	13.6	2.3				12	25.5	5.7
TINKER	LF	9/17/96	SUMMER	R	12	0.115	0.010	12	6768	920	12	60.3	5.4	12	15.1	3.1	12	40.3	10.5	12	15.1	3.5
TINKER	LF	9/17/96	SUMMER	T	12	0.127	0.013	12	4277	444	12	74.9	2.6	12	17.1	3.1	12	38.5	14.2	12	28.8	5.3
TINKER	LF	2/18/97	WINTER	R	12	0.075	0.007							12	20.9	4.0	12	18.2	4.1	12	15.3	6.0
TINKER	LF	2/18/97	WINTER	T	12	0.084	0.007	12	14903	1217	12	58.2	3.4	12	16.2	2.0	12	15.6	3.1	12	15.4	3.0
DBL EAGLE	SP	9/21/96	SUMMER	R	12	0.111	0.007	12	6773	664	12	64.2	3.5	12	22.1	7.9	12	15.9	7.5	12	19.3	8.2
DBL EAGLE	SP	9/21/96	SUMMER	T	12	0.098	0.010	12	8751	939	12	53.8	5.0	12	18.2	3.9	12	12.6	5.6	12	11.3	2.0
DBL EAGLE	SP	2/22/97	WINTER	R	12	0.073	0.007	0			0			12	19.3	2.8	12	32.2	6.2	12	22.7	6.5
DBL EAGLE	SP	2/22/97	WINTER	T	11	0.079	0.006	0			0			12	15.1	2.6	12	25.8	7.9	12	8.5	1.7
LT= Landtreatment																						
SP=Sludge Pit																						
LF= Landfill																						
AP=Asphalt Pit																						

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 AP=Asphalt Pit
 R=Reference Site
 T = Toxic Site
 DTH=Delayed Type Hypersensitivity
 DPM=Disintegrations Per Minute
 NKcell=Natural Killer Cell
 Con-A=Concanavalin A
 PWM=Pokeweed Mitogen
 IL-2=Interleukin-2

Table 1^h. Delayed type hypersensitivity, splenic natural killer cell activity (apoptosis, % specific lysis), and splenocyte proliferation following stimulation with Con-A, PWM, and IL-2 of adult cotton rats collected from reference and toxic sites in Oklahoma, USA during September 1995 through March 1998.

LOCATION	TYPE	DATE	SEASON	SITE	DTH		NKcell		NKcell		Splenocyte Proliferation											
					Absolute Difference		Apoptosis		Apoptosis		Slim Ind.		Slim Ind.		Slim Ind.							
					n	x	stderr	n	x	stderr	n	x	stderr	n	x	stderr	n	x	stderr			
TULSA SECT E	LT	9/29/96	SUMMER	R	12	0.090	0.007	12	4938	452	12	67.4	3.0	12	10.1	1.9	12	71.5	16.9	12	34.2	7.3
TULSA SECT E	LT	9/29/96	SUMMER	T	12	0.063	0.010	12	5078	919	12	66.5	6.1	11	17.7	6.5	11	73.3	21.8	11	41.0	10.8
TULSA SECT E	LT	2/26/97	WINTER	R	12	0.081	0.006	8	15959	2509	8	68.3	5.0	12	28.4	3.7	12	42.0	11.2	12	24.5	3.9
TULSA SECT E	LT	2/26/97	WINTER	T	12	0.075	0.004	12	19331	1651	12	61.7	3.3	12	43.8	7.2	12	40.5	5.1	12	26.5	4.6
TULSA CAP	SP/LT	9/25/96	SUMMER	R	12	0.096	0.011	12	7269	539	12	68.1	2.4	12	39.9	9.7	12	61.2	17.4	12	32.6	4.2
TULSA CAP	SP/LT	9/25/96	SUMMER	T	12	0.088	0.009	12	6776	410	12	70.2	1.8	12	49.6	14.6	12	67.8	15.3	12	44.0	10.2
TULSA CAP	SP/LT	3/2/97	WINTER	R	12	0.075	0.006	12	9637	651	12	77.6	1.5	12	9.1	1.5	12	21.3	4.0	12	14.4	2.5
TULSA CAP	SP/LT	3/2/97	WINTER	T	12	0.077	0.006	12	8828	608	12	79.5	1.4	12	6.4	0.5	12	15.0	2.3	12	10.4	1.5
DUNCAN LF	LT	9/20/97	SUMMER	R	12	0.110	0.010	12	4961	403	12	75.2	2.0	12	7.7	1.2	12	9.8	1.9	12	10.8	2.0
DUNCAN LF	LT	9/20/97	SUMMER	T	11	0.103	0.007	12	4615	474	12	76.9	2.4	12	8.9	1.9	12	7.0	1.1	12	8.7	1.0
DUNCAN LF	LT	2/17/98	WINTER	R	12	0.072	0.007	12	6253	766	12	69.5	3.7	12	11.5	2.8	12	21.1	3.6	12	15.6	3.3
DUNCAN LF	LT	2/17/98	WINTER	T	12	0.073	0.007	12	6867	603	12	66.5	2.9	12	17.8	2.0	12	14.3	2.1	12	18.5	5.4
DUNCAN TP	AP	9/16/97	SUMMER	R	12	0.088	0.005	12	4822	501	12	77.9	2.3	12	13.0	1.7	12	5.2	1.2			
DUNCAN TP	AP	9/16/97	SUMMER	T	11	0.075	0.009	12	5066	892	12	76.7	4.1	11	10.4	1.9	11	3.5	0.8	11	5.2	0.8
DUNCAN TP	AP	2/25/98	WINTER	R	11	0.099	0.007	12	6349	1048	12	42.0	7.7	12	15.7	3.0	12	31.4	6.5	12	20.7	2.7
DUNCAN TP	AP	2/25/98	WINTER	T	12	0.090	0.006	11	5603	816	11	46.9	6.6	12	8.2	1.4	12	19.9	2.0	12	18.7	1.8
DUNCAN PB	SP	9/24/97	SUMMER	R	12	0.121	0.008	12	5803	244	12	79.0	0.9	12	8.4	1.3	12	4.0	0.5	12	8.1	1.4
DUNCAN PB	SP	9/24/97	SUMMER	T	12	0.096	0.007	12	5708	410	12	79.3	1.5	12	12.7	1.9	12	4.5	0.6	12	7.1	1.0
DUNCAN PB	SP	2/21/98	WINTER	R	12	0.082	0.004	12	5473	462	12	54.6	4.0	12	13.4	3.9	12	21.7	3.3	12	17.8	3.0
DUNCAN PB	SP	2/21/98	WINTER	T	12	0.101	0.006	12	5049	350	12	56.9	2.4	12	19.9	4.1	12	16.8	3.1	12	24.6	6.4
CONOCO	LT/LF	9/28/97	SUMMER	R	9	0.103	0.007	12	6837	899	12	71.2	3.8	12	12.6	2.1	12	6.9	1.1	12	16.7	4.5
CONOCO	LT/LF	9/28/97	SUMMER	T	12	0.102	0.005	12	6183	422	12	74.0	1.8	12	8.1	1.8	12	6.5	0.9	12	9.9	1.3
CONOCO	LT/LF	3/1/98	WINTER	R	12	0.065	0.006	0			0			12	18.0	4.0	12	37.6	13.2	12	31.8	8.1
CONOCO	LT/LF	3/1/98	WINTER	T	12	0.083	0.007	0			0			12	9.2	2.5	12	23.0	8.0	12	22.9	3.8

LT= Landtreatment
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R=Reference Site
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DTH=Delayed Type Hypersensitivity
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